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Supporting Information

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Analysis of the Magnetic Properties of Nitrogenase FeMo Cofactor by Single-Crystal EPR Spectroscopy**

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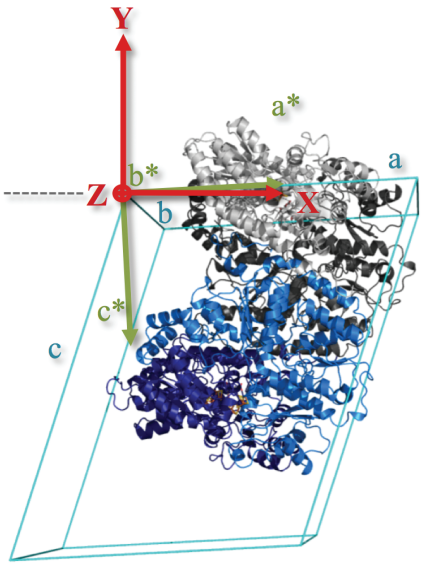
Description of Procedures for g Tensor Alignment



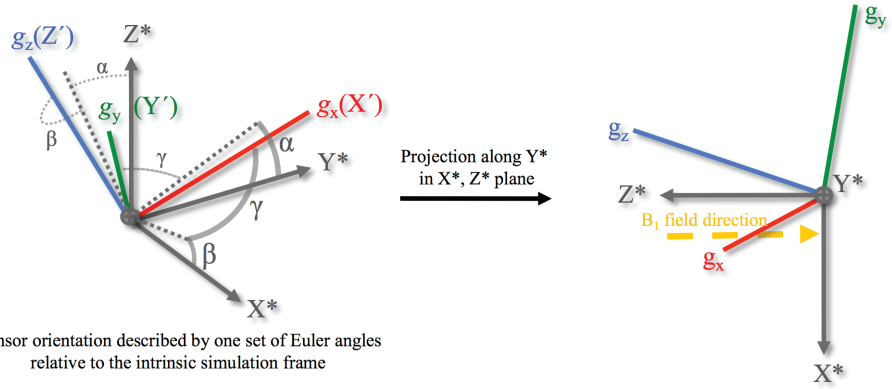
The assignment of **g** tensor orientation required the determination of the relative orientations of three distinct Cartesian coordinate systems:

(i) An **orthogonalized crystal frame** (**a***, **b***, **c***) was derived from the unit cell axes **a**, **b**, **c**.

(ii) An **external reference frame** (**X**, **Y**, **Z**), based on the geometry of X-ray data acquisition. **X** is the direction of the X-ray beam, **Z** is the rotation axis.

(iii) An **intrinsic simulation frame** (**X***, **Y***, **Z***), defined by the direction of the static magnetic field $\mathbf{B}_1$ (**Z***), the rotation axis of the sample (**Y***) and a resulting axis **X*** to complete the right-handed system.

The relation between the intrinsic reference frame and the principal axes of the **g** tensor (g_x(X′), g_y(Y′) and g_z(Z′)) was expressed by sets of three Eulerian angles (α, β, γ) for each magnetically distinct copy of FeMo cofactor. These angles were obtained from the fitting procedure using *easyspin*,[1] and yielded the relative orientations of the corresponding **g** tensors.



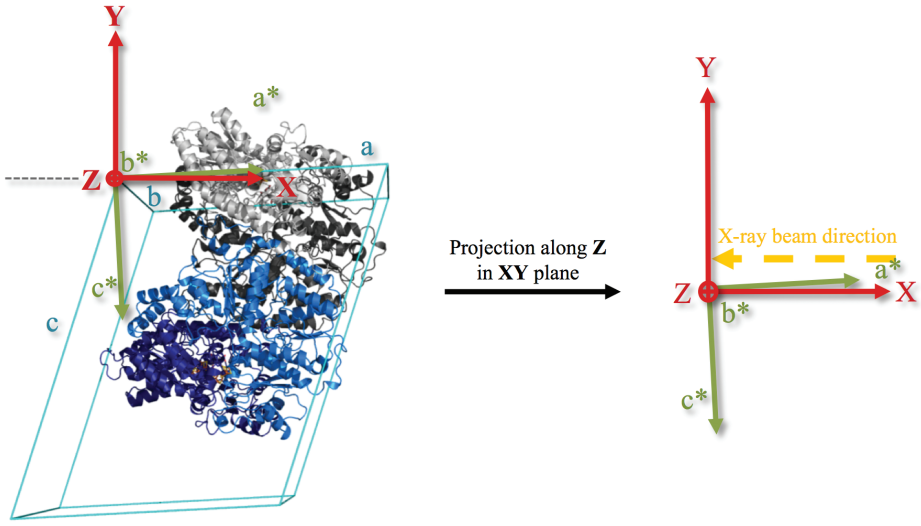
g tensor orientation described by one set of Euler angles relative to the intrinsic simulation frame

At this point the symmetry reduction observed in the second data set (Fig. 3) simplified the evaluation and yielded two sets of Eulerian angles as solutions. The resulting sets of angles were subsequently converted into two direction cosine matrices ($DCM_{1/2}$) using the general expression:

$$\mathrm{DCM} = \begin{bmatrix} \cos\gamma\cos\beta\cos\alpha - \sin\gamma\sin\alpha & \cos\gamma\cos\beta\sin\alpha + \sin\gamma\cos\alpha & -\cos\gamma\sin\beta \\ -\sin\gamma\cos\beta\cos\alpha - \cos\gamma\sin\alpha & -\sin\alpha\cos\beta\sin\alpha + \cos\gamma\cos\alpha & \sin\gamma\sin\beta \\ \sin\beta\cos\alpha & \sin\beta\sin\alpha & \cos\beta \end{bmatrix}$$

As the data acquisition frame (**X**, **Y**, **Z**) and the intrinsic simulation frame (**X***, **Y***, **Z***) shared two axes (sample rotation axis and magnetic field axis / X-ray beam vector), the DCMs could be directly transformed into the data acquisition frame. In the following, this allowed a direct superposition of the **g** tensor and the atomic structure. To this end, the spatial orientation of MoFe protein within the crystal was derived from a single X-ray diffraction image indexed with *mosflm*,[2] yielding an orientation matrix based on the first recorded diffraction image ($\theta = 0°$) of:

$$\begin{pmatrix} a^* \\ b^* \\ c^* \end{pmatrix} = \begin{bmatrix} 0.02039 & 0.00066 & 0.00633 \\ -0.00125 & 0.00039 & 0.01417 \\ 0.00109 & -0.01185 & 0.00082 \end{bmatrix} \begin{pmatrix} X \\ Y \\ Z \end{pmatrix}$$



In the data acquisition frame, the beam direction was designated as **X**, the rotation axis as **Z**. The rotation matrix relating the systems at each point during data collection then provided the correlation between the orthogonalized unit cell (**a***, **b***, **c***) and the X-ray data acquisition frame (**X**, **Y**, **Z**):

$$\begin{pmatrix} a^* \\ b^* \\ c^* \end{pmatrix} = \begin{bmatrix} 0.99673 & 0.05525 & 0.05901 \\ -0.06088 & 0.03282 & 0.99761 \\ 0.05318 & -0.99793 & 0.03608 \end{bmatrix} \begin{pmatrix} X \\ Y \\ Z \end{pmatrix}$$

For each rotation angle, the DCMs (**g** tensor orientation) could then be transformed into the orthogonalized crystal frame (**a***, **b***, **c***). The process of frame alignment and DCM transformations is summarized in Figure S1. The resulting direction cosine matrices ($DCM_{1/2}$**) for the two **g** tensors in MoFe protein with respect to the X-ray data acquisition frame (**X**, **Y**, **Z**) were:

$$\text{FeMoco I} = \begin{bmatrix} 0.25815 & 0.84437 & 0.46947 \\ -0.91794 & 0.36589 & -0.15332 \\ -0.30124 & -0.39137 & 0.86953 \end{bmatrix}$$

$$\text{FeMoco II} = \begin{bmatrix} -0.12975 & 0.43449 & 0.89128 \\ 0.94923 & -0.20535 & 0.23829 \\ 0.28656 & 0.87696 & -0.38578 \end{bmatrix}$$

These matrices describe the relative orientations of the two FeMo cofactors in the asymmetric unit in the Cartesian (**X**, **Y**, **Z**) frame with their corresponding **g** tensors.

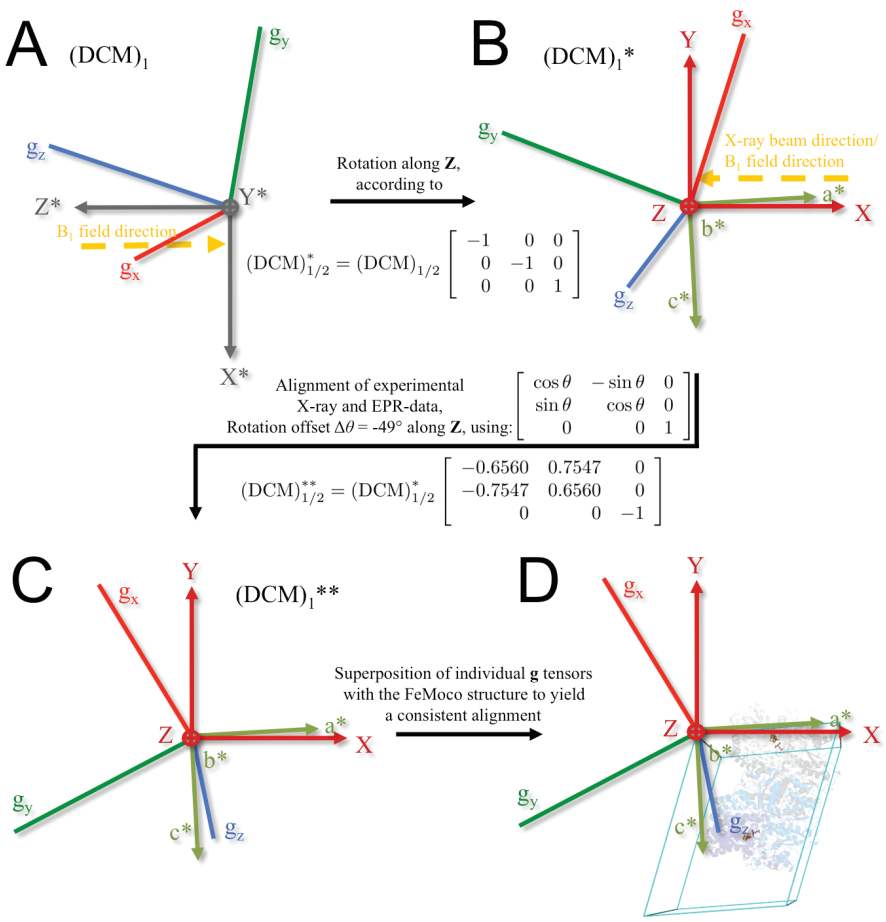


Figure S1: Overview of the alignment process. **A**) Orientation of the **g** tensor with respect to the intrinsic simulation frame (**X***, **Y***, **Z***). **B**) (**X***, **Y***, **Z***) to (**X**, **Y**, **Z**) alignment (matching of crystal orientation with **g** tensor) by rotation around **Z**. **C**) Alignment of X-ray and EPR data (simulation and fit). **D**) Superposition of oriented **g** tensors with spatial coordinates of the three-dimensional structure of MoFe protein.

The simulation (Fig. 3B) resulted in a two-fold ambiguity, due to the fortuitous alignment of a crystallographic axis with the magnetic flied vector $\mathbf{B}_1$. A two-fold ambiguity remained that was subsequently lifted by simulating the spectra of the four independent copies of FeMoco present in a complementary data set (Fig. 2) in which all signals of the four FeMo cofactors in the unit cell were resolved. This then led to a defined **g** tensor orientation with respect to the orientation of each FeMo-cofactor in the unit cell (Fig. 4). For depictions, the **g** tensor was placed in the center of the metal cluster.

Supporting Tables

Table S1: Data collection and refinement statistics

	scEPR crystal	**reference data**[b]
space group	$P2_1$	$P2_1$
unit cell dimensions		
a, b, c [Å]	79.1, 128.6, 107.3	81.2, 130.7, 107.2
α, β, γ [°]	90, 109, 90	90, 109.7, 90
data collection temperature	298 K	100 K
resolution range [Å][a]	52.30 – 2.30 (2.42 – 2.30)	47.50 – 1.00 (1.09 – 1.00)
R_{sym}	0.143 (0.410)	0.100 (0.687)
$R_{p.i.m.}$	0.116 (0.319)	0.042 (0.311)
$I/\sigma[I]$	3.7 (1.7)	8.6 (1.5)
no. of unique reflections	52,965 (8,948)	1,112,854 (252,121)
multiplicity	2.1 (2.2)	6.4 (5.5)
completeness [%]	69.5 (68.7)	99.1 (98.3)
R_{cryst}	0.165	0.128
R_{free}	0.205	0.146
r.m.s.d. bond lengths [Å]	0.017	0.011
r.m.s.d. bond angles [°]	1.810	1.541
average B-factor [Å^2]	27.1	8.1

[a] Numbers in parentheses denote the highest resolution shells

[b] Reference data set from PDB entry 3U7G.

Supporting References

[1] S. Stoll, A. Schweiger, *J. Magn. Reson.* **2006**, *178*, 42-55.

[2] A. G. W. Leslie, H. R. Powell, *Nato Sci Ser Ii Math* **2007**, *245*, 41-51.